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DOI:

[10.1002/mus.26864](https://doi.org/10.1002/mus.26864)

Document Version

Peer reviewed version

[Link to publication record in King's Research Portal](#)

Citation for published version (APA):

Bashford, J., Masood, U., Wickham, A., Iniesta, R., Drakakis, E., Boutelle, M., Mills, K., & Shaw, C. (2020). Fasciculations demonstrate daytime consistency in amyotrophic lateral sclerosis. *Muscle & nerve*.
<https://doi.org/10.1002/mus.26864>

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Full title

Fasciculations demonstrate daytime consistency in amyotrophic lateral sclerosis

Running title

Daytime consistency of fasciculations

Authors

**J Bashford MRCP, PhD¹, U Masood MSc¹, A Wickham PhD², R Iniesta PhD³,
E Drakakis PhD², M Boutelle PhD², K Mills FRCP PhD¹, C Shaw FRCP, MD¹**

¹ UK Dementia Research Institute, Department of Basic and Clinical Neuroscience, Maurice Wohl Clinical Neuroscience Institute, Institute of Psychiatry, Psychology and Neuroscience, King's College London

² Department of Bioengineering, Imperial College London

³ Department of Biostatistics and Health Informatics, King's College London

Corresponding author: J Bashford. Email: james.bashford@kcl.ac.uk. Tel: 020 7848

0259. Website: <https://spiqe.co.uk>

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/mus.26864

Ethical publication statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Conflict of interest statement

None of the authors have potential conflicts of interest to be disclosed.

Word counts

Main text: 2235

Abstract: 148

Keywords

Amyotrophic lateral sclerosis; fasciculation; high-density surface EMG; biomarker; benign fasciculation syndrome; neuronal hyperexcitability

Abstract

Background: Fasciculations represent early neuronal hyperexcitability in amyotrophic lateral sclerosis (ALS). To aid calibration as a disease biomarker, we set out to characterise the daytime variability of fasciculation firing.

Methods: Fasciculation awareness scores were compiled from 19 ALS patients. Additionally, ten ALS patients prospectively underwent high-density surface EMG (HDSEMG) recordings from biceps and gastrocnemius at three time-points during a single day.

Results: Daytime fasciculation awareness scores were low (mean=0.28 muscle groups), demonstrating significant variability (coefficient of variation=303%). Biceps HDSEMG recordings were highly consistent for fasciculation potential frequency (intra-class correlation coefficient [ICC] = 95%, n=19) and the inter-quartile range of fasciculation potential amplitude (ICC = 95%, n=19). These parameters exhibited robustness to observed fluctuations in data quality parameters. Gastrocnemius demonstrated more modest levels of consistency overall (44-62%, n=20).

Discussion: There was remarkable daytime consistency of fasciculation firing in the biceps of ALS patients, despite sparse and intermittent awareness amongst patient accounts.

1 Introduction

Fasciculations are found almost universally in the limb muscles of patients with amyotrophic lateral sclerosis (ALS)^{1 2} and consequently considerable attention has focused on correlating fasciculation parameters with measures of long-term disease progression.³⁻⁵ It is our experience that ALS patients are often unaware of fasciculations until a clinician highlights them. From that time, patients report being most aware of this symptom when relaxed and the muscle is still. It is unknown if this is due to true variability of fasciculation firing during the course of the day or variable levels of attention paid to consistent fasciculation patterns.

This is important for two main reasons. First, the Awaji criteria have emphasised the diagnostic value of fasciculation potentials in the neurophysiological assessment of ALS patients.⁶ It has been proposed that 70-90 seconds are required to confidently exclude the presence of fasciculation potentials using needle EMG⁷ or HDSEMG.⁸ However, an understanding of how diurnal fluctuations might influence these estimates is lacking. Second, this issue will have a bearing on the optimal design of future drug trials that might rely on biomarkers of hyperexcitability or fasciculations to assess outcome.⁹ If there were a particular diurnal pattern to fasciculations, it would be essential to assess patients at a similar time of day.

Only one study has addressed this question, albeit in healthy adults only, showing that there

was significant daytime variability in fasciculation potential frequency in the abductor hallucis longus.¹⁰ Some variability may be attributed to specific daily triggers, for example caffeine consumption, sleep deprivation and recent physical exertion, although the impact of these factors on neuronal hyperexcitability in ALS is poorly understood.^{11 12} Other factors, such as age and stress levels, are likely to influence fasciculation occurrence over longer timeframes.¹³

In this study, we set out to understand how both the subjective awareness and objective measurement of fasciculations vary during the course of the day in ALS patients.

2 Methods

2.1 Patient recruitment for HDSEMG recordings

Ethical approval was obtained from the East Midlands (Nottingham 1) Research Ethics Service (Ref: 17/EM/0221). Patients with ALS were recruited from the King's College Hospital Motor Nerve Clinic between Jun-Dec 2018 and provided informed written consent before participation according to the Declaration of Helsinki. Patients had received a diagnosis of ALS within 24 months of symptom onset, satisfying criteria for probable/definite ALS using the revised El Escorial Criteria.¹⁴ Patients underwent three HDSEMG assessments at 9am, 12pm and 3pm on the same day.

2.2 Data collection and processing

2.2.1 Diary collection

Self-reported diaries were compiled from a parallel longitudinal study of 20 ALS patients.¹⁵ Five of these patients also participated in this current study (see final column of *table 1*). We

asked patients to complete a diary of hourly fasciculation awareness for one week leading up to each assessment, which took place every two months for up to 14 months.

2.2.2 HDSEMG recordings

At the 9am assessment, baseline demographic data and a neurological examination were documented. Patients were asked to report the month of symptom onset (persistent focal weakness). We performed the ALS Functional Rating Scale-Revised (ALSFRS-R)¹⁶ and Medical Research Council (MRC) sum power score (summed total of bilateral MRC power scores for shoulder abduction, elbow flexion, wrist extension, hip flexion, knee extension and ankle dorsiflexion; normal score = 60).¹⁷ Individual MRC power scores of 4+, 4 and 4- were converted to numerical scores of 4.3, 4 and 3.7 respectively. MRC power scores were documented at baseline by the same assessor (UM). All assessments took place in the Academic Neuroscience Centre, King's College Hospital, London, UK.

At each assessment during the day, 30-minute HDSEMG recordings were taken from biceps brachii and medial gastrocnemius on the side of symptom onset. The HDSEMG data collection methods have been previously reported in detail.¹⁸ Briefly, patients were asked to relax on the examination couch with legs in a horizontal, partially flexed position and forearms prone with an elbow angle of 90-120 degrees. Before sensor placement, the skin overlying biceps and gastrocnemius was lightly scrubbed with an abrasive gel and a 70% alcohol wipe. The sensor had 64 circular electrodes (8x8 grid; electrode diameter 4.5mm; inter-electrode distance 8.5mm) and signals were amplified by the Refa-64 EMG Recording System (TMS International BV, The Netherlands). The raw HDSEMG data was stored as a proprietary Polybench file at a

sampling rate of 2048Hz per channel. The sensor was removed in-between assessments on the same day.

Computation of fasciculation potentials was performed in MATLAB (R2014a) using specifically designed scripts and laptops with Intel i7 (2.5GHz) processor. Description and validation of SPiQE's analytical pipeline have been reported elsewhere.¹⁸ In brief, an initial screen for motor unit potentials was applied to each recording channel. This involved the detection of the most extreme amplitudes (positive and negative), representing the peaks and troughs of motor unit potentials. For each of these potentials, the channel with the greatest peak-trough amplitude difference was transferred into a 'super-channel'. Based on manual counts, we found a linear relationship between average noise levels and the optimal amplitude threshold for inclusion of fasciculation potentials. We confirmed that the optimal automated model was a noise-responsive algorithm, capable of adjusting its amplitude inclusion threshold according to the local noise level (referred to as 'noise band'). In addition, areas of the recording with excessive noise were automatically identified and excluded from further analysis. This pipeline achieved a classification accuracy of 88% when applied to 5,318 fasciculation potentials that had been identified manually. Unrelaxed motor unit activity was identified by AVID (Active Voluntary Identification), a semi-automated, flexible system built to exclude regular trains of motor unit potentials.¹⁹ Finally, fasciculation potential parameters for each recording (frequency, amplitude median and amplitude inter-quartile range [IQR]) were computed by SPiQE.

2.3 Statistical analysis

2.3.1 Diary analysis

We converted each hour of diary completion into a fasciculation awareness score (FAS; 0-3), based on the documented awareness of fasciculations: 0 = no report of fasciculations; 1 = fasciculations in one muscle group; 2 = fasciculations in two muscle groups; 3 = fasciculations in at least three muscle groups. Overnight scores (12am-6am) were excluded. Mean hourly FAS was calculated. In order to assess the daytime variability, we calculated the coefficient of variation ($100 \times \text{standard deviation} / \text{mean}$) across each day. These calculations were performed in Prism V7.0a.

2.3.2 Analysis of parameter consistency

In the statistical program R (V3.3.1), we employed a two-way, random, absolute agreement intra-class correlation coefficient (ICC) model to assess the variability of fasciculation potential and data quality parameters between time-points, with 100% reflecting perfect consistency.²⁰ The two-way model was considered the most appropriate, as it treated both the time-points and the assessed muscles as random effects. Estimates of the ICC, alongside 95% confidence intervals, were generated in R using the *icc* function as part of the *irr* package.

3. Results

3.1 Diaries of fasciculation awareness

Diaries from 19 patients were compiled (mean age = 63 years; 17M:2F; mean duration of symptoms = 30 months; site of onset: 37% upper limb/32% lower limb/31% bulbar). A total of 574 patient-days of diary data were collected in blocks of one week. The mean daytime

fasciculation awareness score was 0.28 muscle groups. There was significant diurnal variability with a coefficient of variation of 303% (*figure 1*).

3.2 Variability of fasciculation potential parameters

3.2.1 Data quality

Ten patients with ALS each underwent three assessments at 9am, 12pm and 3pm on the same day (patient characteristics are displayed in *table 1*). For patient 1, recordings from the left biceps were contaminated by the electrocardiogram and were excluded from analysis. The median time included for each recording was 27.7mins (IQR 22.9-29.8mins) after exclusion of unrelaxed motor unit activity and excessively noisy portions of data. The principle measure of data quality (noise band) was extremely variable (ICC did not differ from zero) across the three time-points for both biceps and gastrocnemius (*figure 2d*).

3.2.2 Fasciculation potential frequency (FF)

Half of the patients achieved at least two FF measurements above 50/min in biceps (*figure 2a*). The overall ICC across the three time-points for FF was 88% (95% CI: 76-95%). When each muscle type was analysed separately, biceps achieved remarkable consistency with an ICC of 95% (95% CI: 85-99%), while gastrocnemius was more variable with an ICC of 64% (95% CI: 25-89%). The very high consistency in biceps recordings was particularly noteworthy in the context of extremely variable noise levels.

3.2.3 Fasciculation potential amplitude (median and IQR)

When considering biceps recordings, the ICC did not differ from zero for amplitude median, however it did achieve high consistency for amplitude IQR (ICC = 95%; 95% CI = 87-99%; *figure 2b-c*). Similarly, in gastrocnemius the ICC did not differ from zero for amplitude median but achieved a modest degree of consistency for amplitude IQR (ICC = 46%; 95% CI = 4-82%). Therefore, amplitude IQR was the most robust measure of fasciculation potential across the three time-points, excelling in biceps muscles in a parallel manner to fasciculation potential frequency.

4 Discussion

This study highlights the discord between the subjective awareness and objective quantification of fasciculations in ALS. Although caution is advised when extrapolating beyond a 9am-3.30pm time window, this study sheds light on the daytime variability of fasciculations in ALS patients. The most consistent measures were fasciculation potential frequency and amplitude IQR, and biceps persistently outperformed gastrocnemius. The observed consistency in biceps was particularly striking, considering HDSEMG noise levels have been shown, in this and a previous study,¹⁸ to be significantly higher in biceps than in gastrocnemius. In fact, due to the high variability in noise levels between repeat measurements, this study emphasises the robustness of SPiQE's noise-responsive algorithm. However, it must be noted that extreme noise levels were not managed well. This was demonstrated by the 12pm recording of patient 4 (*figure 2a*), which had the highest noise level recorded in the study (*figure 2d*) and correspondingly produced a FF measurement that differed considerably from the 9am and 3pm recordings.

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These results suggest that the assessment of fasciculations is not influenced significantly by the time of day. Knowledge of this is reassuring in the diagnostic evaluation of ALS patients in the clinical setting and the longitudinal assessment of patients in research studies, imposing no logistical restraints on the time of day that fasciculation assessments take place. A significant contributing factor to the high consistency observed was the duration of each recording. This was set at thirty minutes, longer than other studies assessing fasciculations using surface EMG.^{21 22} Encouragingly, there remained 27.7 minutes per recording after exclusion of voluntary activity and excessively noisy sections of data. Recordings of this duration allowed for any minute-by-minute stochastic fluctuations in fasciculation patterns to be averaged out. This study adds weight to the argument that, in biceps at least, thirty minutes is more than sufficient to record an accurate snapshot of fasciculation potential firing at that time. In fact, shorter durations may be preferred to improve patient convenience, especially if more muscles are being analysed, without detriment to the quality of the analysis. Fasciculations result from neuronal hyperexcitability and their measurement with HDSEMG may prove to be a simple and practical readout of hyperexcitability in clinical trials.⁹

From a theoretical standpoint, it was unsurprising to see that the fasciculation potential amplitude median showed poor consistency across the time-points in the face of highly variable noise levels. Due to SPIQE's noise-responsive algorithm, a shift in noise upwards would lead to a linear increase in the amplitude inclusion threshold. In that scenario, fasciculation potentials in the lower amplitude range would be preferentially excluded, leading to a consequent rightwards shift in the amplitudes of the accepted fasciculation potentials. The median amplitude would be very sensitive to this change, as confirmed in *figure 2*, whereby changes in noise (most notably patients 2, 4 and 6) led to parallel changes in amplitude

median. It was therefore reassuring to see that fasciculation potential amplitude IQR was a much more robust measure, particularly in biceps. This is intuitive, as a rightwards shift in amplitude would be unlikely to alter the spread of amplitudes significantly.

There were several limitations to this study. Only ten patients were included, restricting the generalisability of these results amongst all ALS patients. We did not control for common modifiers of fasciculation potential frequency, such as caffeine consumption. Ideally, we would have liked to take recordings beyond a 9am-3.30pm time window, however the practical limitations of hospital-based assessments made this difficult. We only assessed two muscles, limiting the generalisability of these conclusions to other muscles. It would be particularly relevant to extend this work to muscles that are involved at a relatively early stage of ALS, such as the first dorsal interosseous and tibialis anterior.

In conclusion, this study indicated that fasciculation patterns in ALS patients were consistent throughout the day, particularly so in biceps and often at fasciculation potential frequencies above 50/min. This stood in stark contrast to patient-reported accounts, whereby patients demonstrated a sparse and intermittent awareness of fasciculations. Therefore, when recruiting patients to studies that employ measures of fasciculations or hyperexcitability, it would be inadvisable to screen patients based on their subjective awareness of fasciculations. From a practical standpoint, these results impose no additional logistical burden on the time of day that patients are assessed in clinical and research settings. Moreover, this study further supports the validity of fasciculation potential parameters in the longitudinal assessment of ALS patients as the search continues for reliable disease biomarkers.

Author contributions

JB and UM setup the study and collected and analysed the data. EM, MB, KM and CS supervised the project and provided expert technical and clinical guidance. RI provided expert input on statistical learning. JB wrote the article with editing from co-authors.

Acknowledgements

J. Bashford was supported for 14 months by the Sattaripour Charitable Foundation and the Motor Neurone Disease Association (Shaw/Jul15/932-794). J. Bashford also received support from an MRC/MNDA Lady Edith Wolfson Clinical Research Training Fellowship (MR/P000983/1) between Sep 2016-Sep 2019. A. Wickham was funded by a PhD studentship in the EPSRC Centre for Doctoral Training in Neurotechnology for Life and Health. We would like to thank all the patients involved in this study for their willingness and determination to participate. We thank TMSi for supplying the amplifier and sensors.

Abbreviations

ALS, amyotrophic lateral sclerosis; ALSFRS-R, amyotrophic lateral sclerosis functional rating scale-revised; AVID, active voluntary identification; FAS, fasciculation awareness score; (HD)SEMG, (High-density) surface electromyography; ICC, intra-class correlation coefficient;

MRC, medical research council; NEMG, needle electromyography; SPiQE, Surface Potential Quantification Engine.

References

1. De Carvalho M, Dengler R, Eisen A, et al. Electrodiagnostic criteria for diagnosis of ALS. *Clin Neurophysiol* 2008;119(3):497-503.
2. De Carvalho M, Kiernan M and Swash M. Fasciculation in amyotrophic lateral sclerosis: origin and pathophysiological relevance. *J Neurol Neurosurg Psychiatry* 2017;88(9):773-779.
3. Bokuda K, Shimizu T, Kimura H, et al. Quantitative analysis of the features of fasciculation potentials and their relation with muscle strength in amyotrophic lateral sclerosis. *Neurol Sci* 2016;37(12):1939-1945.
4. Mateen F, Sorenson E and Daube J. Strength, physical activity, and fasciculations in patients with ALS. *Amyotroph Lateral Scler* 2008;9(2):120-1.
5. Vazquez-Costa J, Campins-Romeu M, Martinez-Paya J, et al. New insights into the pathophysiology of fasciculations in amyotrophic lateral sclerosis: An ultrasound study, *Clin Neurophysiol* 2018;129(12):2650-2657.
6. Costa J, Swash M and De Carvalho M. Awaji Criteria for the Diagnosis of Amyotrophic Lateral Sclerosis A Systematic Review. *Archives of Neurology* 2012;69(11):1410-1416.
7. Mills K. Detecting fasciculations in amyotrophic lateral sclerosis: duration of observation required. *J Neurol Neurosurg Psychiatry* 2011;82(5):549-51.

8. Zhou P, Li X, Jahanmiri-Nezhad F, et al. Duration of observation required in detecting fasciculation potentials in amyotrophic lateral sclerosis using high-density surface EMG. *J Neuroeng Rehabil* 2012;9:78.
9. Benatar M, Boylan K, Jeromin A, et al. ALS biomarkers for therapy development: State of the field and future directions. *Muscle Nerve* 2016;53(2):169-82.
10. Van der Heijden A, Spaans F and Reulen J. Fasciculation potentials in foot and leg muscles of healthy young adults. *Electroencephalogr Clin Neurophysiol* 1994;93(3):163-8.
11. Leite M, Orsini M, De Freitas M, et al. Another Perspective on Fasciculations: When is it not Caused by the Classic form of Amyotrophic Lateral Sclerosis or Progressive Spinal Atrophy? *Neurol Int* 2014;6(3):5208.
12. Simon N and Kiernan M. Fasciculation anxiety syndrome in clinicians. *J Neurol* 2013;260(7):1743-7.
13. Fermont J, Arts I, Overeem S, et al. Prevalence and distribution of fasciculations in healthy adults: Effect of age, caffeine consumption and exercise. *Amyotroph Lateral Scler* 2010;11(1-2):181-6.
14. Brooks B, Miller R, Swash M, et al. El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Other Motor Neuron Disord* 2000;1(5):293-9.
15. Bashford J, Wickham A, Iniesta R, et al. The rise and fall of fasciculations in amyotrophic lateral sclerosis. *Brain Commun* 2020 [In Press]
16. Cedarbaum J, Stambler N, Malta E, et al. The ALSFRS-R: a revised ALS functional rating scale that incorporates assessments of respiratory function. BDNF ALS Study Group (Phase III). *J Neurol Sci* 1999;169(1-2):13-21.

17. Dyck P, Boes C, Mulder D, et al. History of standard scoring, notation, and summation of neuromuscular signs. A current survey and recommendation. *J Peripher Nerv Syst* 2005;10(2):158-73.
18. Bashford J, Wickham A, Iniesta R, et al. SPiQE: An automated analytical tool for detecting and characterising fasciculations in amyotrophic lateral sclerosis. *Clin Neurophysiol* 2019;130(7):1083-1090.
19. Bashford J, Wickham A, Iniesta R, et al. Preprocessing surface EMG data removes voluntary muscle activity and enhances SPiQE fasciculation analysis. *Clin Neurophysiol* 2020;131:265-273.
20. Hallgren K. Computing Inter-Rater Reliability for Observational Data: An Overview and Tutorial. *Tutorials in quantitative methods for psychology* 2012;8(1):23-34.
21. De Carvalho M and Swash M. Fasciculation discharge frequency in amyotrophic lateral sclerosis and related disorders. *Clin Neurophysiol* 2016;127(5):2257-62.
22. Kleine B, Stegeman D, Schelhaas H, et al. Firing pattern of fasciculations in ALS: evidence for axonal and neuronal origin. *Neurology* 2008;70(5):353-9.

Figure legends

Figure 1. Heat map representation of self-reported fasciculation awareness amongst ALS patients. 574 patient-days of diary data were collected. Patients provided diary data for

seven consecutive days at intervals of two months. Fasciculation awareness scores (FAS) were applied to each one-hour block during the 24-hour daily cycle: 0=no awareness, 1=fasciculation awareness in one muscle group; 2=fasciculation awareness in two muscle groups simultaneously; 3=fasciculation awareness in 3 or more muscle groups simultaneously. Patient ALS13 did not provide any diary data owing to significant upper limb disability.

Figure 2. Variability in fasciculation potential parameters and noise band amongst ALS patients. Comparison of parameters across three time-points (9am/12pm/3pm) per patient per muscle. ICC estimates displayed. ICC, intra-class correlation coefficient.

Table 1. Patient characteristics.

Patient No.	Age (years)	Gender	Site of symptom onset	Duration since symptom onset (months)	ALSFRS-R at time of assessment	MRC sum power score at time of assessment	Side assessed	Elbow flexion power on side of assessment (MRC scale)	Plantar flexion power on side of assessment (MRC scale)	Corresponding diary data in <i>figure 1</i> (where available)
1	67	M	Bulbar	36	40	60	L	5	5	ALS11
2	80	M	Both ULs	35	33	47.5	R	3	5	ALS13
3	62	M	Bulbar	18	29	48.1	R	5	2	ALS16
4	58	M	Right UL	14	47	55	R	3.7	5	ALS19
5	71	M	Right UL	61	41	55.7	R	3.7	5	ALS20
6	82	F	Left UL	120	41	43	L	3	3	N/A
7	58	M	Bulbar	20	44	60	L	5	5	N/A
8	50	F	Left LL	6	39	51	L	5	3	N/A
9	65	F	Left UL	13	32	48	L	4	5	N/A
10	68	M	Left UL	23	36	42	L	1	4	N/A

ALS, amyotrophic lateral sclerosis; ALSFRS-R, amyotrophic lateral sclerosis functional rating scale-revised; LL, lower limb; MRC, Medical Research Council; UL, upper limb.



